

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application for Research Grant
(Use extra pages as needed)

JAN 30 1974

Date: 1/18/74

1. Principal Investigator (give title and degrees):
 - a) Richard K. Lee, Graduate Student, M.S.
 - b) Shri N. Giri, Assistant Professor of Pharmacology, Ph.D.,
Faculty Principal Investigator.
 - c) Stuart A. Peoples, Professor of Pharmacology, M.D., Faculty Principal Investigator.
2. Institution & address:
School of Veterinary Medicine
University of California
Davis, California
95616
3. Department(s) where research will be done or collaboration provided:
Department of Physiological Sciences
4. Short title of study:
Cigarette Smoke and its Effects, in Man and Animals, on the Metabolism of
the Well Known Pesticide DDT and DDT Metabolites.
5. Proposed starting date: July 1, 1974
6. Estimated time to complete: One year
7. Brief description of specific research aims:

To determine whether cigarette smoking in humans results in lower endogenous levels of DDT and its metabolites, and concurrently to determine whether cigarette smoking enhances the rate of metabolism of exogenous DDT which may enter by the diet and or by intentional oral administration. In both cases to also determine whether a concomitant excretion as DDA, the principal urinary metabolite of DDT, is observed.

To determine the mechanisms involved for the proposed increase in DDT metabolism as a result of exposure to cigarette smoke.

To determine the constituent of tobacco smoke which is responsible for the postulated increase in DDT metabolism in those who smoke. Whether the causative agent of tobacco smoke is nicotine, benzpyrene or some other component of the tobacco smoke.

To determine the feasibility of using Urinary DDA levels to measure the microsomal enzyme induction state of man and animals without altering the homeostatic balance of the experimental subject.

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This author has previously made a finding that there are in fact higher levels of Urinary DDA in those who smoke. It is believed that such an increased level of DDA is a result of an enhanced ability, by those who smoke, to metabolize both exogenous and endogenous DDT and or metabolites of DDT. The result of such an increase in metabolism being the beneficial ability to excrete DDT and or its metabolites, from the body, as Urinary DDA. For this reason, it is believed that a study of the effects of cigarette smoke on the levels of DDT, its stored metabolites and its urinary metabolite, DDA, is indicated.

It is also believed, due to the ubiquitous nature of DDT, that the use of Urinary DDA as a tool for measuring the microsomal enzyme induction state of man and animals should be investigated.

9. Details of experimental design and procedures (append extra pages as necessary)

Experiment 1: Procedure for the determination of the effects of cigarette smoking on the metabolism of DDT and metabolites in man.

Approximately fifty cigarette smokers, of which half will be male and half will be female, will be obtained. Classification will be according to the number of cigarettes smoked per day as well as to the length of time they have been smoking. An equal number of controls will also be selected.

Blood samples and twenty four hour urine samples will be taken. A correlation between blood levels of DDT, DDE and possibly DDD with the urinary levels of DDA will be made and any distinction between smokers and nonsmokers will be noted.

Blood analysis for DDT and metabolites will be by the modified Dale Method (Nachman et al., Health Lab Sci. 6: 148, 1969). Urinary DDA analysis will be determined by the method which made the initial finding, concerning the relationship between cigarette smoking and Urinary DDA levels, possible (Lee et al., Proc. West. Pharmacol. Soc. 16: 240, 1973).

Experiment 2: Procedure for determining the mechanisms involved which account for the observed increased levels of DDA in the urine of those who smoke.

Experiment 2-A: In vivo effects of cigarette smoke on the metabolism of DDT and DDE in rats.

A lighted cigarette will be attached to one end of a six liter chamber made to house four rats. Air will be drawn through the chamber. The rats will be exposed to five cigarettes per hour for four hour intervals each day for a total of two days.

Twenty four hours after the last exposure, 30 mg/kg p,p' DDT will be administered to one group of rats i.p. and 30 mg/kg p,p' DDE will be administered i.p. to another group. An equal volume of solvent will be administered to a control group. Urine will be collected at twenty four hour intervals for two

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days. DDA analysis will be as previously described and a determination of whether cigarette smoke increases the metabolism of either DDT and or DDE should be possible.

Experiment 2-B: In vivo determinations in rats of the effects of cigarette smoke on the metabolism of DDT-¹⁴C and DDE-¹⁴C.

Rats will be exposed to cigarette smoke as previously described. DDT-¹⁴C will be administered i.p. to one group and DDE-¹⁴C to another. Dosage for both isotopes will be 4 mg/kg, Sp. A. 1 mc/mM. Controls will be given an equal volume of the solvent. Urine will be collected for a twenty four hour period and at the end of said period, a venous blood and fat biopsy sample will be taken. Determination of the metabolites will be by thin layer chromatography and subsequent quantitation by scintillation counting (Datta et al., Tox. Appli. Pharm. 6: 321, 1964).

Experiment 2-C: In vitro effects of cigarette smoke on the metabolism of DDT and DDE.

Again, rats will be exposed to cigarette smoke and lung and liver homeogenates will be made (Alary et al., Tox. Appli. Pharm. 18: 457, 1971). Subsequently, p,p' DDE and p,p' DDT will be added to different homeogenates (0.1 ml of 1 mg/ml) and incubated. Analysis for parent compounds and metabolites will be made (Saschenbrecker et al., J. Agr. Food Chem. 15: 168, 1967). A possible inquiry into the use of kidney tissue may be made.

Experiment 3: Procedure for determining the constituent of cigarette smoke which is responsible for the observed increase in Urinary DDA in those who smoke.

The use of known microsomal enzyme inducers, which are known to be present in tobacco smoke, will be utilized in an attempt to elucidate the mechanism involved for the observed increase in Urinary DDA levels in those who smoke.

Initial experiments will be conducted through the use of nicotine and 3, 4-benzpyrene. Rats will have their microsomal enzymes induced by these compounds and experiments analogous, and in a similar step wise manner, to those previously described will be performed.

Rats will be given nicotine sulfate, 2 mg/kg/2 ml s.c. daily for fourteen days. Controls will receive saline, 2 ml, 0.85 g/ml w/v NaCl/kg. Another group of rats will be given 3, 4-benzpyrene, 0.5 ml of 20 mg/kg in corn oil, for one day.

After induction of the microsomal enzymes the following experiments, which have been previously described in more detail, will be attempted.

Experiment 3-A:

Rats whose microsomal enzymes have been induced by either nicotine or benzpyrene will be administered a large i.p. dose of either p,p' DDT or p,p' DDE (30 mg/kg). The urine will be collected for twenty four intervals and analyzed for the presence of DDA.

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Experiment 3-B:

Rats will be administered nicotine and benzpyrene as described. This will be followed by an i.p. administration of DDT- ^{14}C or DDE- ^{14}C , 4 mg/kg, Sp. A. 1 mc/mM. A correlation between DDT and its metabolites in blood, fat and urine by thin layer chromatography and scintillation will be made. A similar analysis will be made for DDE.

Experiment 3-C:

After inducing the microsomal enzymes of the rats with either nicotine or benzpyrene, lung and liver homogenates will be made. Subsequently p,p' DDT and or p,p' DDE (0.1 ml of 1 mg/ml) will be added to the separate homogenates and incubated. Analysis for parent compounds and metabolites will be made. A possible inquiry into the use of kidney tissue may be made.

Experiment 4: Procedure for the determination of whether Urinary DDA levels can be used as a means of measuring the induction state of the microsomal enzymes of man and animals.

Experiment 4-A: The use of humans who are receiving treatment for epilepsy with either phenobarbital or diphenylhydantoin, both being known microsomal enzyme inducers, will be made.

Urine will be collected from out patients in the Epilepsy Clinic, Sacramento Medical Center, Sacramento, California. A correlation between Urinary DDA levels and the administration of phenobarbital and or diphenylhydantoin will be made.

Experiment 4-B: Microsomal enzyme induction in rats and its effects on the Urinary DDA levels.

Rats will be used as the experimental subject placed five per metabolism cage from which their urine will be collected at three hour intervals. Three groups of five rats will be used as controls. Experimentals will be administered pretreatment i.p. doses of phenobarbital each day (75 mg/kg) for one week. Controls will be administered a like volume of saline. Diet will be ad libitum and will be measured. Urine will be collected and analyzed for DDA. Hopefully, a difference in metabolism of dietary DDT will be observed as the enzymes are induced. At random, hexobarbital (125 mg/kg) will be injected into a control and experimental to measure the sleeping time and to delineate whether induction of microsomal enzymes is actually taking place.

At the end of the pretreatment period an i.p. dose of DDT (30 mg/kg) in corn oil will be given to all animals to determine whether there is in fact an increased metabolism of DDT to DDA. A sufficiently large dose of DDT is utilized to eliminate any contribution from endogenous DDT and or metabolites.

Of major significance of this part of the proposal is the possible use of Urinary DDA as a tool for measuring the induction state of the microsomal enzymes of humans. This is of particular importance for patients receiving known microsomal enzyme inducers, or a combination of such inducers.

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That one could test the microsomal enzyme induction state of man or animal, as is; that is without altering the homeostatic balance of the subject (due to the ubiquitous nature of DDT), would seem to be significant contribution of this proposal. This is emphasized when one considers the ease with which Urinary DDA samples can be obtained and the facility with which they can be analyzed (Lee et al., Proc. West. Pharmacol. Soc. 16: 240, 1973).

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

1. Aerograph 1520 Gas Liquid Chromatograph.
2. Walton Horizontal Smoke Exposure Machine.
3. Beckman LS- 200 B Liquid Scintillation Spectrometer.
4. Isco Fraction Collector.
5. Sorvall Model RC 2-B High Speed Refrigerated Centrifuge.
6. Cold room.
7. Warburg Apparatus.
8. Virtis Extracto-matic.
9. Space available includes research laboratories, office space and animal housing.
10. Mettler H-15 Analytical Balance.

11. Additional facilities required:

None required.

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12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

4.

14. First year budget:

A. Salaries (give names or state "to be recruited")

Professional (give % time of investigator(s) even if no salary requested)

Shri N. Giri, Ph. D., Assistant Professor

% time

Amount

11

\$2,975 (including 15% fringe benefits)

Shri N. Giri, Ph. D., Assistant Professor

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Stuart A. Peoples, M.D., Professor

5

\$1,970 (including 15% fringe benefits)

Stuart A. Peoples, M.D., Professor

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*In keeping with policy of the Veterinary Medical Strict Full-time salary plan, 75% of faculty effort associated with the project must be charged to the project.

Technical

Richard K. Lee, M.S., Staff Research Associate, Step II 100

\$11,758 (including 12% fringe Benefits)

Sub-Total for A \$16,703

B. Consumable supplies (by major categories)

Animals (300 CRD Free Rats)

\$1200

Animal Care (Rats; 6¢/day)

150

DDT-¹⁴C, Ring Labeled, 1.0 mCi.

\$1500

Chemicals

100

Regular Lab Supplies

100

Sub-Total for B \$3050

C. Other Expenses (itemize)

Principal Investigators to attend meetings

500

Maintenance and repair charges for existing equipment

500

Sub-Total for C \$1000

Running Total of A+B+C \$20,753

D. Permanent equipment (itemize)

Sub-Total for D --0--

E. Indirect costs (15% of A+B+C)

E \$3,112

15. Estimated future requirements:

Total Request \$23,865

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	\$16,703	\$3040	\$1000	--0--	\$2823	\$23,865

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16. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
1. Physiological Basis For the Genesis of Pulmonary Edema.	Tuberculosis and Respira- tory Disease Association of California	\$ 7570	7/1/73 to 6/30/74
2. Studies on the Avicide 1339.	Starling Research	\$10,000	7/1/73 to 6/30/74
3. Forensic Toxicology	Robert L. King, Associates	\$ 1000	7/1/73 to 6/30/74

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
1. Role of Lung Microsomal Enzymes in Pathogenesis of Chemically Induced Pulmonary Edema and Fibrosis	American Lung Association	\$19,679	7/1/74 to 6/30/75
2. Edema formation and Drug Metabolism in the Lung.	National Institutes of Health	\$219,345	5/1/74 to 4/30/77

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Checks payable to

The Regents of the University of
California
Mailing address for checks
Davis, California 95616

Principal investigator

Typed Name Shri N. Giri, Ph.D.Signature Shri N. Giri Date 21st Jan. 1974Telephone (916) 752-1598

Area Code Number Extension

Responsible officer of institution

Typed Name Allen G. MarrTitle Dean, Research DevelopmentSignature Allen G. Marr Date 1-28-74Telephone (916) 752-0650

Area Code Number Extension

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